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Items of Interest in Seed

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INTRODUCTION

The Seed Regulatory and Testing Branch (SRTB) has published "Items of Interest in Seed Control" for many years. With this issue, we are changing the name to "Items of Interest in Seed" to reflect the broader scope of our activities as well as the broader interests of our readers. In addition to our regular articles relating to seed labeling and testing for enforcement of the Federal Seed Act, we plan to include more information about such items as our international seed activities and service testing program. April 2006 marks the third anniversary of SRTB's move to Gastonia, NC, and information about our expanded laboratory activities in the areas of seed pathology and variety testing are included in this issue. We hope you will find it informative. If you have any comments on this issue of "Items of Interest in Seed" or suggestions for future issues, please send them to Seed Marketing Specialist Linda Vanderhoof (linda.vanderhoof@usda.gov).

MEETING WITH THE CANADIAN FOOD INSPECTION AGENCY

On February 6, 2006, the Agricultural Marketing Service (AMS), Seed Regulatory and Testing Branch (SRTB) hosted a cooperative meeting in Gastonia, NC, with the Canadian Food Inspection Agency (CFIA) seed group. Individuals attending from CFIA were: Glyn Chancey, Michael Scheffel, Louise Duke, Frank Lewis, Krista Thomas, Christine Tibelius, and Dave Warner. Individuals attending from AMS, SRTB were: Randall Jones, Richard Payne, Susan Maxon, Perry Bohn, Jeri Irwin, Pattsy Jackson, and Gene Wilson.

The meeting included many interesting topics to encourage free movement of seed between the United States and Canada including: Organization for Economic Cooperation and Development (OECD) Seed Schemes, and Canada's acceptance of AMS's Accredited Seed Laboratory (ASL) program, which permits the test results from accredited laboratories to be recognized in Canada by Canadian graders.

We also discussed the requirements needed for Canada to recognize individuals working at an ASL as having the ability to grade seed for Canada once they are approved as graders. Potentially, this would allow seed to be tested and labeled in the United States for final delivery to customers in Canada. AMS, SRTB representatives are optimistic that a program can be put in place and that Canada would recognize it as equivalent to their own requirements.

For information regarding this article contact Perry Bohn, OECD Seed Schemes, (704) 810-7262; perry.bohn@usda.gov

SEED SAMPLING WORKSHOPS

Seed Regulatory and Testing Branch (SRTB) Chief Dr. Richard Payne and Seed Marketing Specialists Roger Burton, Kevin Robinson, and Gene Wilson conducted two seed sampling workshops at the SRTB facility in Gastonia, NC, December 6 and 8, 2005. These workshops were available to State seed inspectors and seed certification personnel from North Carolina and nearby States. Several SRTB staff members also attended the workshops.

Ten State seed inspectors from Georgia, North Carolina, and Tennessee attended the December 6 workshop. The purpose of the training was to discuss the proper equipment and procedures for sampling seed in various types of containers and provide guidance in completing inspector sampling report forms to include information helpful for Federal Seed Act (FSA) enforcement. Proper sampling techniques and the appropriate use of various types of triers were demonstrated as part of the training. The work by State seed inspectors contributes to the successful FSA regulatory action against mislabeled interstate seed shipments.

The following topics were covered:

- Seed Inspector's Conduct
- Sampling Procedures
- Planning and Execution of Sampling
- Sampling Documentation
- Sampling Documentation Problems
- Sampling Small Containers
- Sampling Bin and Bag Lots
- Sampling Bulk Containers
- Germination Test Dates
- Replacement Labels

Eleven employees of seed certifying agencies from Georgia, North Carolina, South Carolina, and Virginia attended the December 8 workshop. The purpose of the training was to discuss proper equipment and sampling procedures for seed in different types of containers, the importance of examining primary samples for contaminants, and information that should be included on sampling report forms. The participants sampled bags of seed as part of the training. The requirements of Title V in the FSA and FSA labeling requirements were also discussed.

The following topics were covered:

- Sampling Procedures
- Planning and Execution of Sampling
- Sampling Documentation
- Sampling Slide Presentation
- Sampling Bin and Bag Lots
- Sampling Bulk Containers
- Title V Varieties
- Certification Labeling Issues

For information regarding this article contact Branch Chief Dr. Richard Payne (704) 810-8871; richard.payne2.@usda.gov).

ASSOCIATION OF OFFICIAL SEED ANALYSTS RULES FOR TESTING SEEDS

Six new AOSA rule changes became effective October 1, 2005, and will remain current through September 30, 2006. Changes include the addition of working weights for some species and revisions in uniform classification of some weed and crop seeds. Pure seed definitions were changed for several grass species.

Kinds that are affected by these rules changes for pure seed definitions are: smooth brome (*Bromus inermis*), fairway crested wheatgrass (*Agropyron cristatum*), standard crested wheatgrass (*Agropyron desertorum*), tall wheatgrass (*Elytrigia elongata*), intermediate wheatgrass (*Elytrigia intermedia* subsp. *intermedia*), pubescent wheatgrass (*Elytrigia intermedia* subsp. *intermedia*), western wheatgrass (*Pascopyrum smithii*), hard fescue (*Festuca brevipila*), red fescue *(Festuca rubra*), chewings fescue *(Festuca rubra* subsp. *commutata*), sheep fescue (*Festuca ovina*), and hair fescue (*Festuca tenuifolia*). Under the AOSA Rules, these kinds now require a caryopsis at least one-third the length of the palea in order to be considered pure seed. This had already been part of AOSA's pure seed definition for meadow fescue (*Festuca pratensis*), tall fescue (*Festuca*

arundinacea), and the ryegrasses (*Lolium* spp.) since 2003. However, the Federal Seed Act (FSA) regulations still consider these kinds to be pure seed if a caryopsis with some degree of endosperm development

can be detected. Until such time that the FSA regulations may be amended, we will take the AOSA rule changes into consideration whenever any of these kinds are submitted to us as alleged complaints under the FSA.

In addition, eight AOSA by-law changes were adopted, one of which establishes an outline for the voting rights of the Society of Commercial Seed Technologists (SCST) members on AOSA rules. Within the AOSA and SCST membership, a two-thirds cumulative majority vote is required to adopt a rule proposal. The other seven by-law changes pertained to administrative details and will not directly influence seed testing procedures.

Botanist Pattsy Jackson is a member of the AOSA Rules Committee. For more information about this article, contact her by phone at 704-810-8883 or e-mail at pattsy.jackson@usda.gov. Information about the AOSA Rules is available on their web site at (http://www.aosaseed.com/).

LABELING REQUIREMENTS FOR CHEMICALLY TREATED SEED

There are three issues to be considered when labeling chemically treated seed: (1) labeling the treatment name, (2) labeling seed treated with a chemical not assigned to Toxicity Category I by the Environmental Protection Agency (EPA), and (3) labeling seed treated with a Toxicity Category I chemical.

Section 201(a)(1) of the Federal Seed Act (FSA) and Section 201.31a of the FSA regulations contain requirements for labeling chemically treated seed. Section 201.31a(a) of the FSA regulations requires agricultural and vegetable seeds that are chemically treated to be labeled with the name of the treatment. Examples of appropriate labeling include, "Treated with (name of substance or process)" or "(name of substance or process) treated." Section 201.31a(b) of the FSA regulations requires the name of the treatment to be the "commonly accepted coined, chemical (generic), or abbreviated chemical name." Examples of appropriate names include thiram and captan. Therefore, "Treated with Thiram" or "Thiram Treated" would be appropriate labeling.

In addition, Section 201(a)(d) of the FSA regulations requires seed treated with a chemical, other than a mercurial or a chemical with similar toxicity, to be labeled with "Do not use for food," "Do not use for feed," "Do not use for oil purposes," or "Do not use for food, feed, or oil purposes," if the amount remaining with the seed is harmful to humans or other vertebrate animals. The most commonly used labeling for seed with these types of seed treatments is "Treated with (name of substance)" and "Do not use for food, feed, or oil purposes."

We recently received a question about FSA labeling requirements for seed treated with low levels of a Toxicity Category I chemical. Section 201.31a(c)(1) of the FSA regulations states "Seed treated with a mercurial or similarly toxic substance, if any amount remains with the seed, shall be labeled to show a representation of a skull and crossbones at least twice the size of the type used for information required to be on the label under the paragraph (a) and shall also include the red letters on a background of distinctly contrasting color a statement worded substantially as follows: "This seed has been treated with Poison," "Treated with Poison," "Poison treated," or "Poison." The word "Poison" shall appear in type no less than 8 point. Products assigned Toxicity Category I on the basis of oral, inhalation, or dermal toxicity by the EPA regulations for labeling of a product are considered similarly toxic to mercurials. This means that if a chemical is sold in a package or container labeled with a skull and crossbones and the word "Poison," seed to which this chemical has been applied must also be labeled with a skull and crossbones and the word "Poison" even

though the amount of the chemical applied to the seed is minimal. Appropriate labeling for seed treated with Toxicity Category I chemicals would be "Treated with (name of substance)" and "Poison" with a depiction of a skull and crossbones.

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PACKAGE CLAIMS OF 99.99 PERCENT WEED FREE

The Seed Regulatory and Testing Branch (SRTB) has observed during its routine reviews of packaging claims that some seed companies opted to include on their containers the statement, "**Guaranteed 99.99% Weed Free**."

Although in many cases this statement may be accurate, it is possible a laboratory test could result in findings that would be out of tolerance with the 99.99 percent weed-free statement.

If this should be the case, corrective action would require both the relabeling of the containers and corrective action regarding the container statement **"Guaranteed 99.99% Weed Free."** In the worst-case scenario, the inability to correct the statement could even cause the containers involved to be removed from the marketplace.

The SRTB considers this to be an easily avoided hardship if the companies currently using this packaging technique would consider using the claim of 99.90 percent rather than 99.99 percent. This position is easily justified because current Association of Official Seed Analysts rules and Section 201.47(c) (3) of the Federal Seed Act Regulations require that, if any component in the purity examination is determined to be present in any amount calculated to be less than 0.015 percent, then that component shall be reported as 0.01 percent.

In other words, if only one weed seed is found, no matter how small the weighed amount, it shall be reported as no less than 0.01 percent.

For information regarding this article contact Seed Marketing Specialist Roger Burton (704) 810-7265; roger.burton@usda.gov

QUESTION AND ANSWER

Question: Is Thickspike Wheatgrass Subject to the Federal Seed Act (FSA)?

Answer: Yes and no! Thickspike wheatgrass is not in the list of agricultural seeds subject to the FSA Regulations (section 201.2 (h)), but 'Critana' thickspike wheatgrass is included in the FSA certification standards (section 201.76, footnote 50). The scientific name for thickspike wheatgrass is *Elymus lanceolatus* subsp. *lanceolatus*. Slender wheatgrass is also *Elymus lanceolatus* subsp. *lanceolatus* and **is** a kind subject to the FSA. To understand how a single species could include more than one kind of seed, it is helpful to recall that a similar situation occurs with *Zea mays* in which one species includes three different kinds in the FSA: field corn, pop corn, and sweet corn.

For information regarding this article contact Laboratory Supervisor/Assistant Chief Susan Maxon (704) 810-8877, susan.maxon@usda.gov

TRIERS, PROBES, AND AUTOMATIC SEED SAMPLERS--PART III

In Parts I and II of this article, which appeared in previous issues of the Items of Interest in Seed Control, we discussed triers, probes, and other seed sampling equipment used for random sampling

and referenced in domestic and international sources of information. In Part III, we conclude the article with a discussion of stream flow sampling with particular emphasis on automatic seed samplers.

Stream Flow Sampling

In order to avoid the need for manual sampling or damaging seed bags or containers, sampling seed while containers are being filled is a viable option. In fact, the International Seed Testing Association (ISTA) recommends such sampling when possible as an efficient and effective sampling method.

As opposed to random sampling from bags of seed, this method utilizes systematic sampling, or the drawing of samples at regular intervals from the flow of seeds. ISTA considers systematic sampling to offer a more representative sample of the quality of the seed than does random sampling. This is true provided that certain conditions are met during the sampling process.

- Samples should be taken as the last step before seeds enter their container.
- The cross section of the seed stream should be uniformly sampled.
- Seeds must not bounce out of the sampler.
- The seed must not be damaged nor selected according to some trait such as seed size or chaffiness.

Automatic Seed Samplers

Sampling, as described above, may be performed manually or by devices controlled by timers, called automatic seed samplers. Such samplers draw off a portion of the seed stream and are generally composed of a sampling unit, the outlet for the removal of the sample, and the holding container. It is best if the sampler can be easily inspected and cleaned.

Automatic seed samplers come in various shapes and sizes and have almost limitless variations. In general, there are four basic types, although within each type, considerable variation can still occur. ISTA lists these four types as follows:

1. The entire seed stream is diverted by a two-way valve.
2. A box connected with an outlet moves in and out of the seed stream.
3. The seed stream is diverted over a fixed beak connected to an outlet.
4. A moving beak connected to an outlet passes through the cross-section of the stream.

As with manual sampling equipment, not all samplers for stream sampling are acceptable. Screw samplers and rotary tube samplers do not fulfill ISTA requirements and cannot be used to obtain samples for purposes of issuing ISTA International Seed Analysis Certificates (ISTA Certificates).

Because of the wide variety of automatic seed samplers, ISTA Rules for Seed Testing include none of them. However, approved automatic seed samplers can be used for issuing ISTA Certificates. The reader is referred to Section 5.3.2.1 of the ISTA Handbook on Seed Sampling for more detailed information.

Unlike manual sampling with a trier, using an automatic seed sampler requires adjusting the seed sampler to obtain a representative seed sample. Factors such as rate of flow of the seed and the size of the lot will determine the setting of the sampler for the amount of time between samples.

It is important to note, however, two important points. The first is that the automatic sampler should not be readjusted once a lot of seed is being run, and second, the rate of flow of the seed must be

constant once it has begun. In other words, the timer is set for a specific rate of flow; changing the timing or the rate of flow will alter the results of the sampling. If automatic seed samplers are not set correctly -- for instance, if the sampling interval is too wide -- then the quality of the seed sample will be diminished accordingly.

In addition to the earlier reference to ISTA's approval process for automatic seed samplers, we must take note of the "Protocol for automatic seed sampling and the accreditation of it" as accepted by the ISTA Bulking and Sampling Committee in 1998 and as it appears in Table A.5.10 of the ISTA Handbook on Seed Sampling. There are several points in the handbook, which seem to represent a common sense approach to the use of automatic seed samplers even for laboratories not seeking accreditation or approval. The following points are especially noteworthy:

- The seed flow must be constant and the entire cross section of the seed stream must be sampled uniformly.
- The sampler may not be adjusted during the sampling of a specific lot.
- The sampler must have a closed and uninterrupted duct to the sample container.
- The sampler and related parts should be easily cleaned.
- The company is responsible for the proper adjustment and operation of the automatic sampler.
- Ten lots should be sampled by both automatic sampler and manually to determine if the automatic seed sampler is giving similar results.
- The company should keep records of the adjustment of the sampler in addition to the normal sampling information such as lot number, etc.
- The sampler should be checked for accuracy at least once each year.

Conclusion

The quality of laboratory results can be directly dependent upon the quality of the original seed sample. The most modern laboratory equipment or the most meticulous laboratory procedures will be insufficient if the seed sample is taken with faulty or improper equipment or inappropriate procedures. The sample submitted for testing must be representative of that lot of seed.

To improve the quality of seed sampling we are trying to provide a vital understanding of the wide variety of available sampling tools, including automatic seed samplers, and how to use them correctly.

For information regarding this article contact Seed Marketing Specialist Gene Wilson (704) 810-8888; gene.wilson@usda.gov

PLANT PATHOLOGY AT SRTB

Of the many host-pathogen combinations related to seed health, only some have standardized protocols for detection. The Federal Seed Act (FSA) Regulations includes testing methods for *Neotyphodium* species (fungal endophyte) in certain species of grasses and the microbiological assay for fungicide-treated seeds per the Association of Official Seed Analysts (AOSA) Handbook No. 26. The International Seed Testing Association (www.seedtest.org) rules include validated standard methods for seed health testing and currently cover protocols for twenty pathogens of international interest. The Seed Regulatory and Testing Branch (SRTB) Plant Pathology Laboratory primarily conducts fee-for-service testing that involves testing for the pathogen requested by the customer.

When a request for a pathology test is not covered by existing seed testing rules, then SRTB Plant Pathologist Sandra Walker must find an acceptable protocol to use for testing. The National Seed Health System (NSHS) and the International Seed Health Initiatives (ISHI) have standard protocols available online.

The ISHI (www.worldseed.org) protocols are the result of efforts by the International Seed Federation to develop internationally accepted standard protocols for important diseases of vegetable, herbage, and field crops. The SRTB Plant Pathology Laboratory has tested watermelon (*Citrullus lanatus*) seeds for *Didymella bryoniae* using the ISHI protocol.

The NSHS (www.seedhealth.org) is a USDA-Animal and Plant Health Inspection Service (APHIS) program established to provide consistent methods for seed health testing within U.S. laboratories. The NSHS protocols cover important diseases of both vegetable and field crops in the United States. The SRTB Plant Pathology Laboratory has tested for *Fusarium* using the NSHS protocols for *Fusarium moniliforme* on corn (*Zea mays*). The customer request was to test for *Fusarium* on sorghum-sudangrass (*Sorghum bicolor* x *S. sudanense*). As no protocols for sorghum-sudangrass are available, the SRTB Plant Pathology Laboratory adapted the test using both the blotter method and the culture plate method developed for corn. The SRTB Plant Pathology Laboratory is currently evaluating the efficacy of these protocols for use with sorghum-sudangrass.

The SRTB Plant Pathology Laboratory also completed tests on rice seeds grown in North Carolina for a producer who wanted to export the seeds to another country where the quantity could be increased. Representatives from APHIS Plant Protection and Quarantine in Raleigh, NC asked the SRTB to conduct the requisite tests for pathogens before issuing a phytosanitary certificate.

In addition, the SRTB Plant Pathology Laboratory has adapted the Baermann funnel method to detect nematodes on such seeds as onion (*Allium* spp.) and tall fescue (*Festuca arundinacea*).

The most recent issues in seed pathology have been bacterial fruit blotch of cucurbits caused by *Acidovorax avenae* subsp. *citrulli* and pepino mosaic virus in tomato (*Lycopersicon esculentum*).

For information regarding this article contact Plant Pathologist Sandra Walker (704) 810-7268; sandra.walker@usda.gov

AGRONOMY LABORATORY ACTIVITIES

In a previous issue of the Items of Interest in Seed Control (IOI), we introduced Dr. Michael Lovelace as the Seed Regulatory and Testing Branch (SRTB) Agronomist. Below is information on some of Dr. Lovelace's projects that include developing and conducting tests for distinguishing traits among varieties.

Method Development

One aspect of trait differentiation is using bioassays to detect the presence of Roundup Ready™ (RR) crops. Currently we have been working with RR alfalfa, a relatively new technology that has received regulatory approval by the Food and Drug Administration, Environmental Protection Agency, and U.S. Department of Agriculture for sale and growth in the United States. The presence of the RR gene in alfalfa has created concern among some alfalfa growers. For domestic alfalfa growers, correct labeling of RR alfalfa and possible presence of RR seeds as off-types in traditional varieties have become concerns. For export alfalfa growers, the potential of RR contamination has created greater trade barriers. Scientists at the SRTB have initiated tests to distinguish RR alfalfa from conventional alfalfa (non-transformed alfalfa), but tests are still in the development stage. These tests consist of bioassay, immunoassay, and Polymerase Chain Reaction (PCR) techniques

for detecting and quantifying the presence of the RR trait in alfalfa seeds. The SRTB is focusing on developing a rapid and accurate bioassay method. The current test from Forage Genetics International and Monsanto Company to determine RR alfalfa requires growing out the seeds in a greenhouse or growth chamber and spraying the seedlings with the herbicide. This method not only requires equipment that most State laboratories cannot access, but is also a very lengthy test (28 to 35 days). SRTB's preliminary tests focus on substrate imbibition and are conclusive within 7 to 10 days. In addition, we are working with the RR alfalfa test strips for detection of the RR trait. We will have more information on these tests in a future issue of the IOI.

The SRTB is also involved in developing other tests. Older seed may pose challenges for certain crops such as soybeans, where age greatly reduces seed vigor. Conducting an herbicide bioassay on low vigor soybeans to detect the RR gene can be difficult. Soaking the seeds in Roundup™ solution can cause abnormalities in RR soybean seedlings that mimic susceptible soybean seedlings. We have found that if low vigor soybean seeds are imbibed on blotter paper saturated with Roundup™ solution (1700 PPM) for two hours and then transferred to towels wetted with water, the damage to the seed from soaking is minimized greatly. In a seed soak, the seeds appear to undergo tremendous stress when submerged in the herbicide solution, and many cells are being damaged in the process. In addition, initiating the test in herbicide-soaked towels appears to affect seedling growth. This may be due to the effective herbicide contact area from the towels and constant exposure of the seeds to the herbicide solution. By limiting the seed and herbicide contact area and exposure time to the herbicide, less stress is imposed on the seed and fewer cells are subject to herbicide damage.

Since RR and *Bacillus thuringiensis* (Bt) technologies are relatively common, there are methodologies developed for detecting the presence of these traits. The introduction of new seed technology pertaining to the Federal Seed Act has been limited in previous years, thus many of our efforts have been aimed at promoting uniform seed testing rather than the new method development. Over the past few years, the SRTB has conducted training to detect biotechnology traits in seeds. Through Dr. Lovelace's participation in these workshops, he has observed a need for accurate and uniform herbicide mixing instructions for solutions used in germination-based bioassay tests.

Dr. Lovelace has developed an interactive herbicide mixing and calculation spreadsheet to assist laboratories in these tasks. Our plans are to make this spreadsheet available on our Web site. Through meetings and training sessions, we hope more people become aware of the inaccuracies in the current methods and learn how to properly use the new spreadsheet. Once this spreadsheet is up and running, we will post information and directions in a future issue of the IOI.

Variety Testing

Another aspect of SRTB's agronomy work is to develop and conduct varietal purity tests based on grow-outs, either in the field or in growth chambers. A large part of our varietal purity tests consists of trueness-to-variety (TTV) field testing. For our TTV tests, we obtain samples from the State seed laboratories and through direct order from seed companies. We organize the samples by kind and each is grown at a site with which the SRTB has a cooperative agreement to conduct the TTV tests. Visual observations are made throughout the growing season to determine if the samples are correctly labeled. Test samples are compared with check samples, which are collected from various entities. If a test sample is questionable and thought to be mislabeled, visual results can be validated through various physiological tests, including electrophoresis. SRTB Plant Physiologist Dr. Yujia Wu conducts the physiological tests.

The SRTB also receives submitted samples for varietal purity testing not included in the TTV tests. These samples are grown in growth chambers in an environment where a multitude of plant

characteristics can be evaluated. Varieties are not always distinguishable under normal growing conditions. If we cannot detect differences among varieties under normal growing conditions, we will look at the variety descriptions to see if the variety has resistance or tolerance to some sort of stress. For example, some varieties may have tolerance to high heat or salt. If this is the case, we grow the varieties in the presence of these stresses and then observe the phenotype of the varieties under these stresses to determine any visual differences. Again, the Agronomy Laboratory works together with the Plant Physiology Laboratory to conduct physiological tests that may elucidate differences among varieties. In the near future, the SRTB will also have a greenhouse to facilitate these tests.

The Agronomy Laboratory has the opportunity to conduct both field and controlled growth studies. The major differences between field and growth chamber variety tests are the sizes of the tests and the environment. In the field, a greater number of plants can be screened than is possible in a controlled environment. However, in the field, the plants are at the mercy of Mother Nature. Field conditions can be quite variable from day to day, as well as from year to year. Insects, weeds, and pathogens may affect only some parts of the field. Soil type and fertilizer application may not always be uniform across the field. There are many unknown and unpredictable factors present in field situations, but these factors may also be responsible for elucidating differences among varieties. Growth chamber tests are much smaller and conditions predictable, controlled, and repeatable over time. The environment is easily manipulated in the growth chambers, which is why growth chamber tests are desirable. Both controlled growth chamber tests and uncontrolled field tests have pros and cons, but the two tests conducted together provide a very effective testing program for the detection of mislabeled seed.

The testing carried out by the Agronomy Laboratory are generally in support of our Federal Seed Act enforcement program but are also available as part of our fee-for-service testing.

For information regarding this article, contact Agronomist Dr. Michael Lovelace (704) 810-7261; michael.lovelace@usda.gov

PLANT PHYSIOLOGY LABORATORY INTRODUCTION

The activities of the Plant Physiology Laboratory of the Seed Regulatory and Testing Branch (SRTB) center primarily on the development or refinement of methods for variety testing. Dr. Yujia Wu, SRTB Plant Physiologist, has concentrated on protein electrophoresis methods in such crops as tomato, tall fescue, and wheat. This article provides background on the methods and how they are being applied at the SRTB.

Plant physiology and protein electrophoresis

Plant physiology is the study of the function, or vital processes, of plants. Fundamental processes such as photosynthesis, respiration, and protein characterization, are studied by plant physiologists. Protein, an important molecular component of all living cells, is composed of long chains of amino acids that have the generic formula R-CH (NH_3)+ COO-. There are many thousands of different kinds of protein, all with unique amino acid sequences. Because of the varying lengths and structures of the amino acid chains, proteins differ in both electrical charge (isoelectric point) and size (molecular weight). Both of these parameters are utilized in electrophoretic separations.

There are many types of electrophoresis techniques used for protein separation. Two common methods are SDS-PAGE (Sodium Dodecyl Sulphate – PolyAcrylamide Gel Electrophoresis) and IEF (Iso-Electric Focusing) native gel electrophoresis. Because crops have different types and amounts of protein, the differences may be characterized using electrophoresis, and the resulting characterizations are used to identify various crop varieties. Electrophoresis is a rapid, accurate, laboratory method for crop variety identification, compared with traditional agronomist field testing.

Crop seed structure
Most crop seeds consist of three parts: a dormant embryo, storage tissue, and a seed coat. Different plants have different types of seed structure. Plants are classified as monocots or dicots based upon the number of seed leaves (cotyledons) in the seed. In monocot (one seed leaf) crop seeds, such as wheat, corn, or barley, the endosperm is retained as the storage tissue; while in dicot (two seed leaves) crop seeds, such as soybean, cotton, or sunflower, the storage materials are retained in the cotyledons. Starch, protein, and oil, are main storage materials in crop seed as well as energy sources for human use. The various types and amounts of protein are important indexes used for characterizing and analyzing crop seed quality. As these protein differences represent genetic differences, once those differences are characterized, they can be used for variety testing.

PART I: SDS PAGE Gel

A commonly used type of protein electrophoresis is SDS-PAGE (Sodium Dodecyl Sulphate – PolyAcrylamide Gel Electrophoresis). In this method, all gel buffers, tank buffers, and sample buffers include SDS, which is a powerful anionic (negatively charged) detergent. SDS can bind protein and form negatively charged molecules. When SDS-treated proteins are run through a gel using SDS-PAGE, protein molecules are separated by size in the gel medium. Smaller protein molecules move through the gel faster than larger ones.

Many researchers report that large crop seeds, such as wheat, barley, oat, and corn, have a large amount of storage protein in their endosperm tissue, making it easy to extract and separate by SDS-PAGE. Several traditional methods, for example 2-Chloroethanol and DiMethyl Formamide (DMF), have been used for storage protein extraction. Also, special detergent-based reagents such as SDS denaturing buffer may be used for rapid and efficient protein extraction in various types of seed.

In order to improve the resolution of protein band patterns in PAGE, Dr. Wu recently introduced a new method of protein extraction using phenol. These studies using the new method focused on two aspects of the SDS-PAGE procedure. During protein extraction, a phenol solvent with SDS Tris pH 7.5 buffer was used for wheat seed protein extraction. All soluble protein and some ethanol-soluble protein were extracted into the phenol phase, and then precipitated in methanol overnight at -20 °C. For comparison with other extraction methods, a one-step, crude extraction was used and the protein was loaded directly onto the gel. This phenol extraction resulted in a selective precipitation for pure protein only, minus starch, nuclease, and other compounds. In addition, the normal uniform-percentage SDS-PAGE gel was replaced by a linear gradient SDS-PAGE gel that enhanced the separation of similar molecular weight proteins. Based on these changes in procedure, it was possible to obtain high-quality SDS-PAGE gels with highly resolved protein bands. The result is a rapid and high-quality method that can be used for seed protein isolation and subsequent seed variety examination.

A future article will describe SRTB's work with Iso-Electric Focusing (IEF).

For more information regarding this article, contact Plant Physiologist Dr. Yujia Wu, 704-810-7267; yujia.wu@usda.gov.

GROWTH CHAMBER TESTS OF COLLARDS AND RAPE

At the Seed Regulatory and Testing Branch (SRTB), we work closely with the State Seed Laboratories to enforce the Federal Seed Act. In the past few months, the SRTB has received several complaint samples from various State Seed Laboratories concerning contamination of collards with rape. In our attempt to acquire check samples, the SRTB went to several seed companies. Samples submitted by the various seed companies also appeared contaminated. Pure seed for check samples was finally received from a plant breeder.

Through growth chamber growouts, SRTB Agronomist Dr. Michael Lovelace confirmed that these samples were contaminated. He found that differences become evident at about 10 to 14 days after emergence. The leaves of collards are dark blue green, have a smooth texture, no hairs present, and a waxy bloom. The rape leaves range in colors from light to medium green, have rougher texture than collards, prominent hairs on the base of the plant and leaf margins, a few hairs on the adaxial surface of the leaf, and no waxy bloom. In addition, the leaf shapes of the two crops differ. SRTB Plant Physiologist Dr. Yujia Wu is currently developing a method using electrophoresis to distinguish between collards and rape.

States that receive collard samples should watch closely for this problem. If collard samples are submitted to your laboratory and you are suspicious that the sample may be contaminated with rape, the SRTB encourages you to submit those samples to us. We plan to conduct a trueness-to-variety trial with collards as well as with other greens in the fall.

For information regarding this article contact Agronomist Dr. Michael Lovelace (704) 810-7261; michael.lovelace@usda.gov

UPDATE ON VARIETY NAME WEB SITE

Although there is no variety name registration system in the United States, in order to help prevent conflicts and violations of the Federal Seed Act, the Seed Regulatory and Testing Branch will review proposed variety names submitted to us prior to marketing. To facilitate this process, we have recently revised some of our Web sitesrelated to variety names (http://www.ams.usda.gov/lsg/seed/varietyname.htm).

We have recently updated and moved the Variety Name Check Application online form from the Name of New Variety and Release Date Section to the Variety Name Check Section. This form can be used when requesting a final check for a variety name prior to the release or marketing of the new variety.

The Name of New Variety and Release Date form remains in the Name of New Variety and Release Date Section. The form can be used to notify us of the name used when you release a new variety and the month and year of release. The notification will allow us to make the variety name a permanent record in our variety name database.

The variety name lists can be accessed through our Web site (http://www.ams.usda.gov/lsg/seed/varietyname.htm) and will be updated every 30 days to keep them current.

Things to consider when submitting a Variety Name Check Application:

- Be sure your company has searched the name(s) on our Web site database.
- Be sure to submit only name(s) of varieties your company plans to market.
- Be sure to include all information needed to issue a clearance letter (name, company name, company address, city, state, zip code, phone number, e-mail address, kind, and variety name(s)).

Things to consider when submitting a Name of a New Variety and Release Date Application:

- Be sure that your company contacts our office when you have chosen a release date for your cleared variety name(s). This should be within 12 to 18 months of the date your clearance letter was issued.
- Be sure to include the specific month and year for the release date.

Things to be aware of:

- Variety names cannot be reserved for later use.
- Allow up to two weeks for clearance letters.
- If a variety name has been cleared for use by one company, this does not guarantee that the name cannot be used by another company first. If a second company requests clearance for a name previously cleared for another company but not reported as used, the name will be cleared for the second company and the name of the original company will also be provided to the second company.

For information regarding this article contact Seed Marketing Specialist Kevin Robinson (704) 810-7264; kevin.robinson2@usda.gov

ADMINISTRATIVE CHANGES

Horticulturist Al Burgoon retired from the Seed Regulatory and Testing Branch (SRTB) in January after more than 20 years of federal service. Operating from an office in Beltsville, MD, Al coordinated SRTB's variety program, including trueness-to-variety (TTV) grow-outs and variety name clearance. With Al's retirement, the office in Beltsville, MD has closed and, at this time, the horticulturist position will not be filled. SRTB's variety program is now consolidated with all SRTB activities in our Gastonia, NC, location. Dr. Michael Lovelace, Agronomist, will coordinate the TTV grow-out program, and Kevin Robinson, Seed Marketing Specialist, will coordinate maintenance of the variety name database and variety name clearances. Please see the Directory of Services for their contact information.

Ernest Allen has been selected to fill the vacant botanist position. Ernest has been with the SRTB as a biological laboratory technician since February 2004. Ernest received his BS in biology from Winthrop University and is currently pursuing a MS in biology from Winthrop University.

Information Technology Specialist Sean Sabo joined the SRTB staff as a permanent employee in January 2006. Sean had been working for the branch as a student trainee/intern since May 2004. He completed his B.S. degree in Computer Information Systems in December 2005 and is currently persuing a Master's degree in Computer Science through James Madison University. Please see the Directory of Services for contact information.

For information regarding this article contact Branch Secretary Karen Sussman (704) 810-8871; karen.sussman@usda.gov.

CALENDAR OF EVENTS

Federal Seed School **Tifton, GA**	**May 9-11, 2006**
Joint Annual Meeting of the Association of Official Seed Analysts (AOSA), the Association of Official Seed Certifying Agencies (AOSCA), and the Society of Commercial Seed Technologists (SCST) Marriott Hotel, Indianapolis, IN	June 2-8, 2006
International Seed Testing Association (ISTA) Annual Meeting 2006 and Executive Committee Meeting Glattbrugg/Zurich, Switzerland	June 25-30, 2006
Joint Annual Convention of the American Seed Trade Association (ASTA) and the Canadian Seed Trade Association (CSTA) Hyatt Regency, Chicago, IL	July 8-12, 2006
Association of American Seed Control Officials (AASCO) Annual Meeting Billings, MT	July 22-27, 2006
Federal Seed School **Texas**	**August TBD**
Joint Annual Meeting of the American Society of Plant Biologists (ASPB) and the Canadian Society of Plant Physiologists Boston, MA	August 5-9, 2006
Organization for Economic Cooperation and Development (OECD) Seed Schemes Annual Meeting Fortaleza, Brazil	August 7-12, 2006
2006 Northeast Seed Analysts Workshop Harrisburg, PA	September 20-21, 2006
Seed Inspector Workshops **Host locations**	**TBD**
Variety Testing Workshops **Gastonia, NC**	**TBD**

Seed Regulatory and Testing Branch (SRTB)-sponsored training is shown in **bold**.
Various SRTB employees will participate in the other meetings.

For further information regarding the Calendar of Events contact Secretary Karen Sussman (704) 810-8871; karen.sussman@usda.gov

DIRECTORY OF SERVICES

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Regulatory Section Fax (704) 852-4109
Testing Section Fax (704) 852-4189

FEDERAL SEED ACT CASES SETTLED

The following cases were settled administratively under the Federal Seed Act between October 1, 2005, and March 31, 2006. Under the administrative settlement procedure, the Seed Regulatory and Testing Branch and the firms agreed to settle the cases for the amount specified, with the firms neither admitting nor denying the charges. Official Program Announcements on each of these cases is accessible on the following Web site: (http://www.ams.usda.gov/news/newsrel.htm):

- Allied Seed, LLC, Nampa, ID, has paid $3,325 for a case involving four seed shipments. The alleged violations, while not the same for all shipments, were false labeling as to germination, pure seed, other crop seed, and inert matter percentages, kind name, and date of test; failure to label as a mixture and to label the presence of noxious-weed seeds; and failure to keep and/or supply a complete record of the seed. Seed regulatory officials in Georgia, Kentucky, and Virginia cooperated in the initial sampling and inspection.

- Barenbrug USA, Tangent, OR, has paid $4,200 for a case involving six seed shipments. The alleged violations, while not the same for all shipments, were false labeling as to germination, pure seed, other crop seed, and inert matter percentages, date of test, kind name, and variety name; failure to label the presence of noxious-weed seeds; and failure to keep and/or supply a complete record of the seed. Seed regulatory officials in Alabama, Kentucky, Missouri, Texas, and Virginia cooperated in the initial sampling and inspection.

- BWI Companies, Inc., Nash, TX, has paid $1,000 for a case involving four seed shipments. The alleged violations, while not the same for all shipments, were false labeling as to germination, pure seed, and inert matter percentages; and failure to label the presence of noxious-weed seeds. Seed regulatory officials in Arkansas, Florida, and Texas cooperated in the initial sampling and inspection.

- Lesco, Inc., Silverton, OR, has paid $1,875 for a case involving four seed shipments. The alleged violations, while not the same for all shipments, were false labeling as to germination, pure seed, other crop seed, and inert matter percentages, noxious-weed seeds, and date of test. Seed regulatory officials in Florida, Georgia, Texas, and Virginia cooperated in the initial sampling and inspection.

- Livingston Seed, Inc., Columbia, OH, has paid $1,375 for a case involving five seed shipments. The alleged violations, while not the same for all shipments, were false labeling as to germination percentages and failure to keep and/or supply a complete record of the seed. Seed regulatory officials in Indiana cooperated in the initial sampling and inspection.

- Pennington Seed, Inc., Madison, GA, has paid $28,325 for a case involving 30 seed shipments. The alleged violations, while not the same for all shipments were, false labeling as to pure seed, other crop seed, and germination percentages, noxious-weed seeds, kind name, variety name, and test date; failure to label the presence of noxious-weed seeds; shipping seed containing noxious-weed seed in excess of State's limits; failure to attach labels; and failure to keep required records, including those establishing kind and variety name. Seed regulatory officials in Alabama, Georgia, Kentucky, and Texas cooperated in the initial sampling and inspection.

- Plantation Products, Inc., Norton, MA, has paid $1,375 for a case involving nine seed shipments. The alleged violations, while not the same for all shipments were, false labeling as to germination standard and as to variety name; and failure to test for germination prior to

interstate shipment and to keep and/or supply a complete record of the seed. Seed regulatory officials in Indiana, Minnesota, Nebraska, and Texas cooperated in the initial sampling and inspection.

- Texas Oklahoma Production Company, Enid, OK, has paid $3,850 for a case involving seven seed shipments. The alleged violations, while not the same for all shipments, were false labeling as to pure seed and other crop seed percentages, noxious-weed seeds, and test date; and failure to keep and/or supply a complete record of the seed. Seed regulatory officials in Arkansas, Georgia, and Texas cooperated in the initial sampling and inspection.

- Turner Seed, Inc., Lavergne, TN, has paid $2,375 for a case involving five seed shipments. The alleged violations, while not the same for all shipments, were false labeling of germination, pure seed, other crop seed, weed seed, and inert matter percentages, test date, and variety name; failure to label the presence of noxious-weed seeds and noxious-weed seeds considered undesirable grass seed; and failure to show required information for a seed component and the shipper's code or name and address. Seed regulatory officials in Kentucky, Ohio, and Virginia cooperated in the initial sampling and inspection.

- Western Productions, Inc., Woodburn, OR, has paid $1,575 for a case involving three seed shipments. The alleged violations, while not the same for all shipments, were false labeling as to pure seed, other crop seed, and inert matter percentages, and test date; failure to label the presence of noxious-weed seeds (undesirable grass seeds); and failure to keep and/or supply a complete record of the seed. Seed regulatory officials in Missouri and Virginia cooperated in the initial sampling and inspection.

RYEGRASS FLUORESCENCE LIST

The current ryegrass fluorescence list by the National Grass Variety Review Board is available on the following Web site: http://www.oscs.orst.edu/publications/specialreports/vfl.pdf

PLANT VARIETY PROTECTION CERTIFICATE STATUS

Check the status of certification and search for expired certificates by accessing the Plant Variety Protection Office's Web site and entering their Public Access Database: http://www.ams.usda.gov/science/pvpo/PVPindex.htm.

"Though I do not believe that a plant will spring up
where no seed has been, I have great faith in a seed.
Convince me that you have a seed there,
and I am prepared to expect wonders."

Henry D. Thoreau; Faith in a Seed, Island Press, 1993

(Contributed by Sandy Dawson; Botanist, Federal Seed Laboratory.)